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Effect of High-Pressure Processing and Milk on the Anthocyanin Composition and Antioxidant Capacity of Strawberry-Based Beverages

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ABSTRACT: The present study investigated processing strategies and matrix effects on the antioxidant capacity (AC) and polyphenols (PP) content of fruit-based beverages: (1) strawberry powder (Str) + dairy, D-Str; (2) Str + water, ND-Str; (3) dairy + no Str, D-NStr. Beverages were subjected to high-temperature−short-time (HTST) and high-pressure processing (HPP). AC and PP were measured before and after processing and after a 5 week shelf-life study. Unprocessed D-Str had significantly lower AC compared to unprocessed ND-Str. Significant reductions in AC were apparent in HTST- compared to HPP-processed beverages (up to 600 MPa). PP content was significantly reduced in D-Str compared to ND-Str and in response to HPP and HTST in all beverages. After storage (5 weeks), AC and PP were reduced in all beverages compared to unprocessed and week 0 processed beverages. These findings indicate potentially negative effects of milk and processing on AC and PP of fruit-based beverages.

KEYWORDS: *anthocyanin, ORAC, antioxidant capacity, high-pressure processing, LC-MS/MS*

INTRODUCTION

A diet rich in fruits and vegetables is associated with reducing the risk of chronic diseases, including cardiovascular disease and some cancers.[1] These effects have been attributed to the essential nutrients they provide and, more recently, polyphenolic compounds known for their antioxidant, anti-inflammatory, and antitumor properties. Strawberries (*Fragaria* spp.) are high in polyphenolic compounds, especially anthocyanins, which give strawberries their red color.[2] Strawberry extracts from freeze-dried fruit as well as their individual components have demonstrated antioxidant/anti-atherogenic activity in vitro and in animal cell culture models.[3] In humans, consumption of strawberry has been shown to reduce lipids,[4] protect low-density lipoprotein (LDL) from oxidation,[5] and attenuate meal-induced inflammation coincident with improved insulin sensitivity,[6] suggesting a significant role of strawberries in chronic disease risk reduction.

Strawberries in fresh form are preferred; however, their delicate tissue and high moisture content present issues for perishability, which limits availability and fresh strawberry consumption. Methods for preserving the "fresh-like" attributes of strawberries, including flavor and nutritional value, have several advantages. Importantly, successful preservation strategies could offer consumers another source of strawberries (nutrients) that is convenient and flexible with different lifestyles. However, using conventional preservation/processing methods that involve higher temperatures (thermal processing) alters the levels of anthocyanins and other phenolic and antioxidant compounds, resulting in reduced antioxidant capacity and potentially reduced bioactivity.[7] Therefore, technologies or processing strategies to reduce the negative impact of thermal exposure may be useful. Among the nonthermal approaches, high-pressure processing (HPP) is regarded as one of the most promising processing techniques for delivering sustained/high nutritional value and excellent organoleptic attributes while meeting food safety requirements.[8,9]

Strawberry purée is often used in the preparation of beverages/shakes with milk to increase product nutritional appeal and taste. However, studies with tea and blueberries suggest an interaction between phenolic compounds and milk proteins rendering the combination less "bioactive" than if the fruit were consumed alone.[10] Because processing may alter polyphenolic−protein interactions, testing the effect of processing on strawberry beverages with and without added dairy (milk) was an objective of this study. Specifically, this study aimed to evaluate the impact of conventional and high-pressure processing, with and without dairy components, on the antioxidant capacity and anthocyanin composition of strawberry-based beverages. A secondary aim was to assess the effects of storage on antioxidant capacity, anthocyanin content, and microbial safety of strawberry beverages after 5 weeks of storage in refrigerated conditions.

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MATERIALS AND METHODS

Freeze-dried strawberry powder was obtained from the California Strawberry Commission (Watsonville, CA). Reagents including 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), sodium fluorescein, potassium phosphate monobasic, potassium phosphate dibasic, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxlyic Acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium acetate·3H_2O, glacial acetic acid, sodium hydroxide, hydrochloric acid (HCl), 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride (FeCl·6H_2O), methanol, and acetone were procured from Fisher Scientific (Pittsburgh, PA). Ingredients for the preparation of the strawberry beverages were purchased from a local market (Jewel Osco, Chicago, IL). For the ORAC assay, a 96-well black microplate (OptiplateF, Perkin-Elmer, Waltham, MA), Envision fluorescence plate reader (Perkin-Elmer, Waltham, MA), and centrifuge (Eppendorf, Hauppauge, NY) were used. Pelargonidin-3-*O*-glucoside, quercetin-3-*O*-glucoside, and kaempferol-3-coumaroylglucoside were purchased from Chromadex Inc. (Irvine, CA). All solvents used for LC-MS analysis were of LC-MS grade and were purchased from Fisher Scientific.

Table 1. Formulation Recipes for Different Beverages[a]

ingredient	ND-Str	D-Str	D-NStr
strawberry Nesquik syrup[b] (g)		34	34
skim milk powder[c] (g)		21	22.5
distilled water (g)	295	237	238.5
white granulated sugar[d] (g)		3	10
freeze-dried strawberry powder[e] (g)	10	10	
total volume (mL)	305	305	305

[a]ND-Str, beverage (freeze-dried strawberry powder + distilled water); D-Str, beverage (freeze-dried strawberry powder + dairy); D-NStr, beverage (all ingredients excluding freeze-dried strawberry powder). [b]Nesquik strawberry artificially flavored syrup, Nestle USA, Inc., Glendale, CA. [c]Premium Sanalac nonfat dry milk, Con Agra Foods, Omaha, NE. [d]Domino sugar, Domino Foods, Yonkers, NY. [e]Freeze-dried strawberry powder, California Strawberry Commission, Watsonville, CA.

Beverage Formulation and Processing. The strawberry beverages were prepared using the recipes shown in Table 1. The dairy beverage (without strawberry) was prepared from nonstrawberry ingredients (D-NStr) to match the flavor, energy, and nutrient profile without the phytochemical composition of the strawberry plus dairy/milk containing beverage (D-Str). Strawberry-based beverages were prepared with freeze-dried strawberry powder and dairy/milk product (D-Str) or water (ND-Str). Strawberry beverages (50 mL each) were sealed in PET/LLDPE pouches no. 400 (Kapak Co, St. Louis Park, MN). With reference to food safety issues and commercial applications of HPP, pressures above 200 MPa with 15 min of holding time were tested with the following results. Beverages were subjected to HPP at pressures ranging from 200 to 800 MPa for different holding times of 1−15 min as shown in Table 2 (sample initial temperature <10 °C and final temperature maintained at range of 18−22 °C) using the 1.8 L HPP unit (Quintus Food Processing cold iso-static press model QFP-6; ABB Autoclave Systems, Inc., Columbus, OH). High-temperature−short-time processing (HTST, 72 °C/20 s) was carried out in a temperature-controlled shaking water bath (Jeio Tech, BS-31, Des Plaines, IL) using 15 mL plastic tubes (VWR International, Wayne, PA). The beverage samples were held at this temperature until they had reached a temperature of 72 °C for 20 s. The temperature was monitored using a digital temperature needle probe (−50 to +300 °C, Fisher Scientific) to ensure accurate inner temperature of the beverage. An agglomerated/denatured mass was observed in response to pressures at 700 and 800 MPa (holding times ≥ 5 min), and therefore these pressures were omitted in the data analysis. Freshly prepared unprocessed and processed samples were analyzed for antioxidant capacity (oxygen radical absorbance capacity, ORAC; and ferric reducing antioxidant power, FRAP), anthocyanin content, and microbiological counts. Additional beverage samples were stored at refrigerated conditions (~4 °C, no exposure to light) for 5 weeks for shelf stability studies. The refrigerator temperature was monitored daily. Once per week for 5 weeks, unopened samples were removed for antioxidant, anthocyanin, and basic microbial analyses.

Table 2. Experimental Design for HPP Conditions

pressure (MPa)	holding time (min)	process temperature (°C)
200	15	18−22
600	1/15	18−22
700	1/15	18−22
800	1/15	18−22

Determination of Antioxidant Capacity. Antioxidant capacity was determined by ORAC and FRAP assays according to the methods of Prior et al.[11] and Benzie and Strain,[12] respectively, both with minor modifications. The ORAC assay is highly detailed and sensitive; it measures the capacity of an antioxidant compound in a biological matrix to directly bind and neutralize free radicals generated.[13] The total ORAC assay measures the antioxidant capacity of hydrophilic and lipophilic fractions of beverages. ORAC values were determined using standard Trolox concentration (1.5625−25.0 μmol/L). ORAC values were expressed as micromoles of Trolox equivalents (μmol TE/mL). Grape seed extract with a known ORAC value (19355 ± 1548 μmol TE/g) was used as an internal quality control in the ORAC assay.

The FRAP assay is a redox-related colorimetric technique. It measures the ferric complex reducing ability of an antioxidant-rich sample.[13] The change of absorbance is analyzed and used for quantifying the reducing capability of the antioxidant. FRAP values were determined by measuring the change in absorption at 593 nm based on the ferric tripyridyl triazine complex (Fe^{3+}−TPTZ) reducing to Fe^{2+}. A linear regression was constructed for the standards (absorbance versus concentration), and using the regression equation, unknown FRAP values of the samples were measured (units, μmol Fe(II)/L) of the samples.

Analysis of Anthocyanin Composition in Strawberry Beverage Samples by LC-MS/MS. A simple liquid extraction method using acetone/water (70:30, v/v) was performed to extract polyphenolic compounds from beverage samples.[14] Phenolic compounds in filtered (through 0.45 μm PTFE syringe filter) extracts of the beverage samples were identified by retention times relative to external standards and MS^2 fragmentation patterns using LC-MS/MS as reported previously.[6] Commercially available reference standards were used to quantify pelargonidin-3-*O*-glucoside, quercetin-3-*O*-glucoside, and kaempferol-3-coumaroylglucoside. All other compounds were relatively quantified using reference standards with similar structures and fragmentation patterns. Concentrations of polyphenolic compounds were reported as micrograms per milliliter for the test strawberry and dairy beverage samples.

Vitamin C (Ascorbic Acid) Assay. Vitamin C (ascorbic acid) analysis was performed according to the method described by Sanchez Mata et al.[15] Briefly, 2 mL of each sample was extracted with 10 mL of a buffer solution containing dithiothreitol (DTT), potassium phosphate (KH_2PO_4), and metaphosphoric acid (HPO_3). Samples were then diluted with 15 mL of reagent alcohol/water (50:50, v/v) and placed on a mechanical shaker for 18 min. A portion of the upper layer was filtered through a 0.2 μm PTFE syringe filter and analyzed by HPLC. The analysis was performed on Shimadzu Prominence HPLC with UV detection at 260 nm. The mobile phase consisted of 75% acetonitrile and 25% 0.15 mol/L acetate, pH 5.0. Chromatographic separation was obtained using a Luna 3u NH2 100A 150 × 2.0 mm column (Phenomenex, Torrance, CA). The concentration of vitamin C was determined using a standard curve. Ascorbic acid standards (0.625−20 μg/mL) were prepared fresh in a buffer solution (see above) and 50% alcohol solution in a 1:3 ratio.

pH Measurement. The pH values of the beverages were measured using a pH-meter (Thermo Scientific Orion 3-Star Plus, Rochester, NY). The pH-meter was calibrated using standard pH solutions before each measurement.

Microbiological Analysis. Strawberry beverages were formulated in aseptic conditions, and unprocessed samples were analyzed for microbiological growth (including pathogenic strains). Automated microbiological analysis was based on the study by Paulsen et al.[16] Twenty-five grams of sample was added to 225 g of Butterfield's phosphate buffer (BPB) in a TEMPO (bioMérieux, Inc., Durham, NC) stomacher bag to dilute the sample 1:10 and homogenized in the stomacher at 200 rpm for 2 min. The culture medium was reconstituted by adding 3.9 mL of sterile water. Then, 0.1 mL of diluted sample was added to the appropriate culture medium vial to prepare 1/400 inoculated dilution. It was mixed in a vortex-type mixer for 3−5 s. The TEMPO Total Viable Count (TVC) cartridge transfer tube was placed in the inoculated medium, and the card was sealed in the TEMPO filler. The cartridge was read in a TEMPO Reader following incubation at 35 ± 1.0 °C for 46−48 h. For pathogen detection, the samples were analyzed with specialized cartridges with the TEMPO reader on the basis of the most probable number (MPN) method. To supplement the automated assay, a Petrifilm (3M, Minneapolis, MN) plating method was also considered: 1 mL of the beverage sample was pipetted into a 9 mL bottle of sterile diluent. By repeating this procedure, using a fresh sterile pipet each time, higher dilutions were obtained, allowing the reduction of the bacterial population to a countable range. One milliliter of diluent was placed on the Petrifilm plate, and by using a presser, the Petrifilm was pressed for even distribution. The plates were incubated for 48 h at 35 ± 1.0 °C. Colonies formed were red-pink in color, they were counted, and the bacterial population (measured in colony forming units, CFU, per mL) was calculated. Results were expressed as mean CFU per mL ± SD of the counts.

Statistical Analysis. All experiments were replicated (n = 3 or 4), and replicated samples were analyzed for antioxidant capacity, anthocyanins, and TVC. Normality and equal variance tests were performed followed by ANOVA to determine differences between treatment means in multiple groups. Paired data were analyzed using the t test. The Sigma plot 11 statistical (Chicago, IL) program was used to analyze the data. All data are presented as the mean ± SD. Treatment means with $P < 0.05$ were considered to be significantly different.

RESULTS

Effect of Dairy/Milk on the Antioxidant Capacity of Unprocessed Beverages. Significantly higher ORAC values were observed in strawberry-containing beverages compared to control beverage with no strawberry (ND-Str, 27.11 ± 0.46 μmol TE/mL, and D-Str, 18.15 ± 0.81 μmol TE/mL, vs D-NStr, 0.24 ± 0.01 μmol TE/mL, respectively, $P < 0.001$). Analysis by FRAP showed results following a similar trend (ND-Str, D-Str, and D-NStr were 4671.36 ± 2.34, 1867.70 ± 0.93, and 1266.34 ± 0.63 μmol Fe(II)/L, respectively) (Figure 1). Strawberry beverages containing dairy (D-Str) had significantly lower antioxidant capacity as measured by both ORAC and FRAP compared to strawberry beverage without dairy (ND-Str, $P < 0.001$) (Figure 1).

Vitamin C (Ascorbic Acid) and the pH of the Beverages (Unprocessed and Processed). The ascorbic acid concentrations in the unprocessed D-NStr, ND-Str, and D-Str were 12.6 ± 0.6, 73.3 ± 3.7, and 49.9 ± 2.6 μg/mL, respectively. The vitamin C content in D-NStr was not significantly changed with any of the processing conditions used in the study. However, the vitamin C content in D-NStr was significantly lowered in response to high-pressure conditions above 600 MPa for 1 min and HTST compared to unprocessed beverage ($P < 0.05$). In D-Str beverages the vitamin C content was not significantly changed with any of the HPP conditions used. However, the vitamin C content in the HTST-treated D-Str beverage was significantly lowered compared to unprocessed beverage (from 49.9 ± 2.5 to 22.4 ± 1.1 μg/mL, $P < 0.05$). The pH values of the D-NStr, ND-Str, and D-Str beverages were 6.5, 3.7, and 5.1, respectively. There was no significant change in pH values in response to the processing conditions tested ($P > 0.05$).

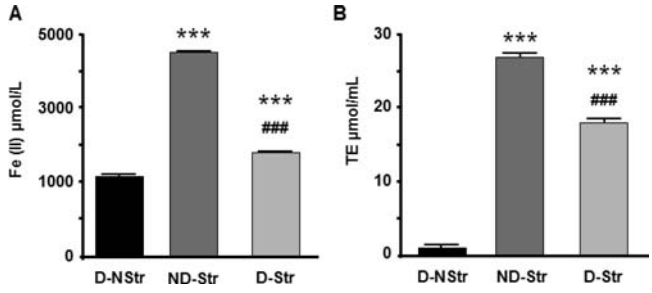

Figure 1. Effect of matrix/dairy on antioxidant capacity of unprocessed beverage samples: (A) FRAP; (B) ORAC values. D-Str, freeze-dried strawberry powder + dairy beverage; ND-Str, freeze-dried strawberry powder + distilled water beverage; D-NStr, dairy (no freeze-dried strawberry powder) beverage. Values represent the mean ± SD; n = 4. (***) $P < 0.001$ compared to D-NStr; (###) $P < 0.001$ compared to ND-Str.

Effect of Processing on the Antioxidant Capacity of Strawberry Beverages. ORAC values of control beverage (D-NStr) were significantly reduced after all processing conditions compared to unprocessed D-NStr ($P < 0.001$) (Figure 2A). No significant changes in ORAC values of ND-Str were apparent when beverages were treated with pressures at 200 MPa for 15 min and at 600 MPa for 1 and 15 min compared to unprocessed ND-Str ($P > 0.05$) (Figure 2B). However, at higher pressures, 700−800 MPa for 1 min, and after HTST processing, ORAC values of ND-Str were significantly reduced compared to unprocessed ND-Str ($P < 0.001$) (Figure 2B). In contrast, strawberry formulations containing dairy/milk (D-Str) resulted in a significant increase in ORAC values when treated with HPP at 200 MPa for 15 min and 600 MPa for 1 and 15 min compared to unprocessed D-Str beverage samples ($P < 0.001$) (Figure 2C). There were no significant changes in ORAC values after HPP at 700−800 MPa for 1 min compared to unprocessed D-Str ($P > 0.05$); however, ORAC values were significantly reduced in response to HTST compared to unprocessed D-Str beverage samples ($P < 0.001$) (Figure 2C). Changes in FRAP are shown in Figure (3). No significant changes in FRAP were apparent for beverages with no strawberry (D-NStr) in response to high-pressure treatment of 200 MPa for 15 min and 600 MPa for 1 and 15 min compared to unprocessed to D-NStr ($P > 0.05$) (Figure 3A). However, FRAP values were significantly reduced when D-NStr samples were subjected to high pressure at 700 and 800 MPa for 1 min and HTST compared to unprocessed D-NStr ($P < 0.001$) (Figure 3A). The antioxidant capacity measured by FRAP in ND-Str was significantly reduced after all processing conditions compared to unprocessed ND-Str ($P < 0.001$) (Figure 3B). No significant changes in FRAP were apparent for beverages with dairy and strawberry (D-Str) in response to pressure treatments at 200 MPa for 15 min and at 600 MPa for 1 and 15 min ($P > 0.05$). In contrast, when HPP at 700 and 800 MPa was applied for 1 min of holding, FRAP was significantly

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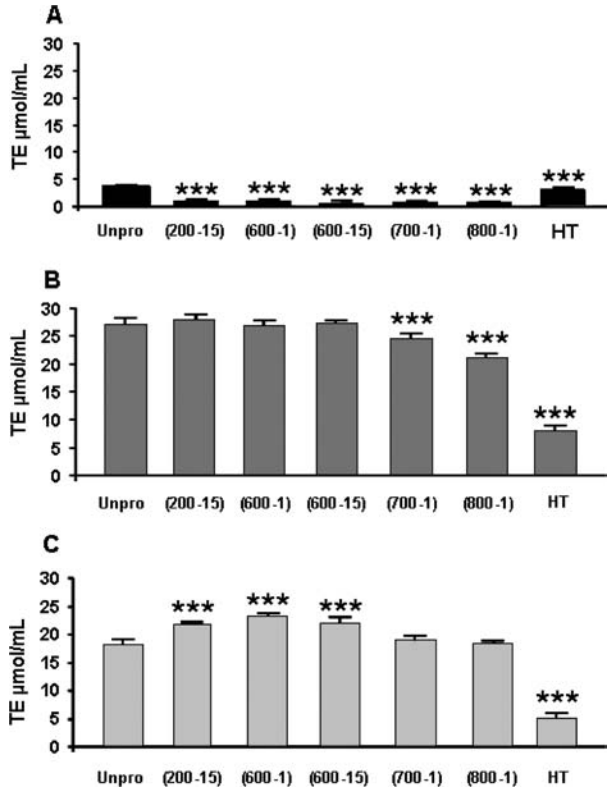

Figure 2. Antioxidant values (ORAC) of beverage samples with different HPP and HTST processing conditions: (A) D-NStr (dairy with no freeze-dried strawberry powder); (B) ND-Str (freeze-dried strawberry powder + distilled water); (C) D-Str (dairy + freeze-dried strawberry powder). Unpro, unprocessed; 200-15 and 600-15, 200 and 600 MPa for 15 min hold, respectively; 600-1, 700-1, and 800-1, 600, 700, and 800 MPa for 1 min hold, respectively; HTST, high-temperature–short time (72 °C/20 s). Values represent the mean ± SD; $n = 4$. (***) $P < 0.001$ compared to Unpro.

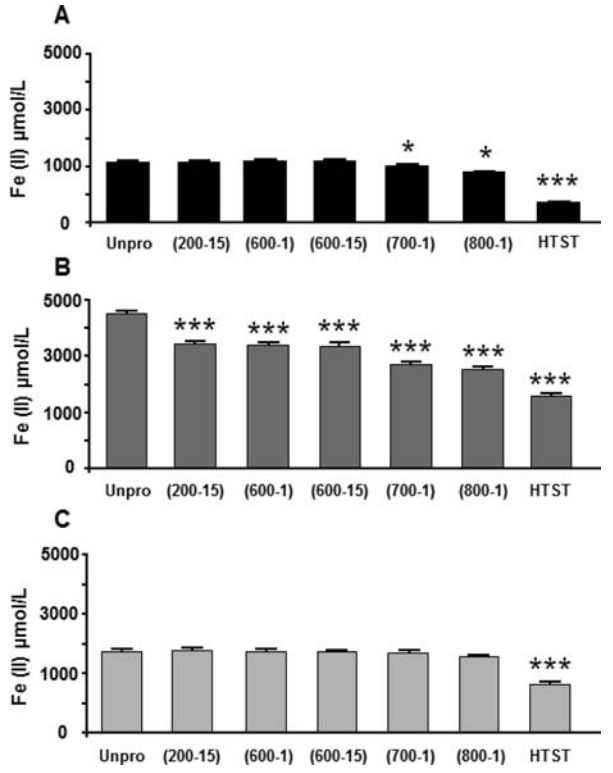

Figure 3. Antioxidant values (FRAP) of beverage samples with different HPP and HTST processing conditions: (A) D-NStr (dairy with no freeze-dried strawberry powder); (B) ND-Str (freeze-dried strawberry powder + distilled water); (C) D-Str (dairy + freeze-dried strawberry powder). Unpro, unprocessed; 200-15 and 600-15, 200 and 600 MPa for 15 min hold, respectively; 600-1, 700-1, and 800-1, 600, 700, and 800 MPa for 1 min hold, respectively; HTST, high-temperature–short time (72 °C/20 s). Values represent the mean ± SD; $n = 4$. (***) $P < 0.001$ compared to Unpro.

reduced in the D-Str beverage sample compared to unprocessed D-Str ($P < 0.001$) (Figure 3C).

Antioxidant Capacity of Strawberry Beverages (Unprocessed and Processed) after 5 Weeks of Storage. All samples were sealed under aseptic conditions and stored in refrigerated conditions (4 °C) after different processing treatments. Irrespective of treatment, the ORAC values of all beverage samples were significantly lower after 5 weeks of storage at 4 °C ($P < 0.05$). After an initial decline in antioxidant capacity that occurred from week 0 (prior to the storage) to week 2, ORAC values remained stable during the second to fifth weeks of storage (data are not shown). Samples processed by HTST compared to HPP had significantly greater reduction in ORAC from baseline to week 5 ($P < 0.05$) (Table 3A).

FRAP values of all HTST-treated beverages were significantly decreased at the end of week 1 of storage compared to FRAP values at week 0 ($P < 0.05$, data are not shown). In contrast, unprocessed and HPP-treated D-NStr and D-Str beverages showed no significant changes in FRAP after 1 week of storage compared to week 0. ND-Str (unprocessed, HPP- and HTST-treated) beverage samples had significantly lower FRAP values at the end of the first week of storage compared to week 0 values (data are not shown). As observed with ORAC, FRAP values were relatively stable during the second to fifth weeks of storage at 4 °C ($P > 0.05$). Significantly reduced FRAP values were observed with HTST processing compared to HPP treatment in all beverage samples at the end of the fifth week compared week 0 (Table 3B).

Effect of Dairy Proteins, Processing, and Storage on the Anthocyanin Composition of Strawberry Beverages (Unprocessed vs Processed). Twenty-seven polyphenolic compounds have been identified in strawberry beverage samples.[6] The most abundant compounds were quantified and are given in Table 4. Pelargonidin-3-*O*-glucoside (PG) along with other flavonoids was not detected in control beverage. The concentrations of PG, pelargonidin-3-malonyl-glucoside, kaempferol-3-coumaroylglucoside, pelargonidin-3-*O*-rutinoside, and quercetin-3-*O*-rutinoside were significantly lower in the unprocessed D-Str compared to unprocessed ND-Str ($P < 0.05$, Table 4). Significant differences observed in polyphenolic compounds in response to HPP (200–800 MPa) and HTST in ND-Str and D-Str samples and their comparisons with related respective unprocessed beverages are given in Table 4.

After 5 weeks of storage, the PG content in the beverages was significantly reduced compared to week 0 in both unprocessed ND-Str (from 53.70 ± 0.3 to 42.74 ± 1.2 µg/mL) and D-Str (from 27.0 ± 0.1 to 16.08 ± 0.7 µg/mL) ($P < 0.05$). However, PG and other estimated polyphenolic compounds in HP-processed D-Str or ND-Str were not significantly different between weeks 0 and 5 ($P > 0.05$). PG concentrations were significantly decreased in HTST-treated beverages after 5 weeks of storage compared to 0 weeks (ND-

Table 3. Reduction (Percent) in (A) ORAC and (B) FRAP Values of Strawberry Beverage Samples after 5 Weeks of Storage Compared to the Samples prior to Storage[a]

	Unpro	200-15	600-1	600-15	700-1	800-1	HTST
				(A) ORAC Values			
D-NStr	39.2 ± 1.2	30.2 ± 0.2	22.0 ± 1.6	29.6 ± 0.7	39.7 ± 2.6	27.8 ± 2.4	70.0 ± 3.1
ND-Str	32.5 ± 0.8	16.0 ± 0.9	26.7 ± 1.0	29.0 ± 2.8	30.9 ± 2.0	26.3 ± 1.9	58.8 ± 3.0
D-Str	26.9 ± 0.1	24.0 ± 2.3	23.8 ± 2.2	43.2 ± 3.0	29.0 ± 3.8	29.3 ± 1.3	45.4 ± 2.8
				(B) FRAP Values			
D-NStr	20.2 ± 1.7	20.6 ± 1.1	19.5 ± 1.8	20.2 ± 2.0	15.9 ± 0.5	15.1 ± 1.1	17.3 ± 1.3
ND-Str	10.4 ± 0.9	16.2 ± 1.4	9.4 ± 0.6	19.7 ± 1.8	9.5 ± 0.7	23.0 ± 2.3	20.8 ± 2.0
D-Str	8.9 ± 0.8	6.3 ± 0.7	9.5 ± 0.9	10.9 ± 0.1	21.5 ± 1.6	27.2 ± 2.5	26.1 ± 2.6

[a]D-NStr, dairy + no strawberry beverage; ND-Str, freeze-dried strawberry powder + distilled water beverage; D-Str, freeze-dried strawberry powder + dairy beverage; Unpro, unprocessed; 200-15 and 600-15, 200 and 600 MPa for 15 min holding, respectively; 600-1, 700-1, and 800-1, 600, 700, and 800 MPa for 1 min holding, respectively; HTST, high-temperature−short time (72 °C/20 s).

Table 4. Polyphenolic Composition of the Strawberry Beverage Samples (Concentrations Are Given in Micrograms per Milliliter)[a]

		pelargonidin-3-*O*-glucoside	pelargonidin-3-malonyl-glucoside	quercetin-3-*O*-glucoside	kaempferol-3-coumaroyl-glucoside	pelargonidin-3-*O*-rutinoside	quercetin-3-*O*-rutinoside	kaempferol-3-malonyl-glucoside	quercetin-3-*O*-malonyl-glucoside
Unpro	D-Str	27.0 ± 0.1*	0.7 ± 0.0*	0.9 ± 0.0*	2.8 ± 0.1*	9.0 ± 0.1*	1.1 ± 0.0*	1.5 ± 0.1*	0.2 ± 0.0*
	ND-Str	53.7 ± 0.5	1.4 ± 0.0	0.9 ± 0.0	4.6 ± 0.2	22.6 ± 2.0	1.5 ± 0.0	0.9 ± 0.1	0.3 ± 0.0
200-15	D-Str	20.3 ± 0.4#	0.5 ± 0.0#	0.6 ± 0.0#	2.6 ± 0.1	11.9 ± 0.6	0.8 ± 0.0	0.7 ± 0.0#	0.1 ± 0.0#
	ND-Str	44.8 ± 0.5#	1.2 ± 0.0#	0.7 ± 0.0	4.4 ± 0.1$	21.6 ± 0.7$	1.4 ± 0.1$	0.5 ± 0.1#	0.2 ± 0.0
600-1	D-Str	20.3 ± 0.4#	0.5 ± 0.0#	0.6 ± 0.0#	2.6 ± 0.1	11.9 ± 0.6$	0.8 ± 0.0	0.7 ± 0.0#	0.1 ± 0.0#
	ND-Str	46.3 ± 1.3#	1.2 ± 0.0#	0.7 ± 0.0	3.5 ± 0.1$	17.2 ± 0.4$	1.2 ± 0.2$	0.7 ± 0.1#	0.1 ± 0.0#
600-15	D-Str	18.2 ± 0.2#	0.5 ± 0.0#	0.8 ± 0.0	2.2 ± 0.0#	12.0 ± 0.3$	0.9 ± 0.1	0.8 ± 0.0#	0.1 ± 0.0#
	ND-Str	46.4 ± 2.1#	1.0 ± 0.2#	0.8 ± 0.0	3.5 ± 0.1$	14.4 ± 0.1$	0.9 ± 0.1#	0.6 ± 0.0#	0.1 ± 0.01#
700-1	D-Str	20.2 ± 0.8#	0.5 ± 0.0#	0.7 ± 0.0	2.3 ± 0.3#	12.2 ± 1.2$	0.8 ± 0.0	0.8 ± 0.0#	0.1 ± 0.01
	ND-Str	41.1 ± 0.8$	1.1 ± 0.0#	0.6 ± 0.0#	3.5 ± 0.1$	15.5 ± 1.5$	0.9 ± 0.0#	0.7 ± 0.0#	0.1 ± 0.01#
800-1	D-Str	18.8 ± 0.2#	0.5 ± 0.0#	0.5 ± 0.0$	2.5 ± 0.1#	14.5 ± 0.0$	0.9 ± 0.1	1.0 ± 0.0#	0.1 ± 0.00#
	ND-Str	39.3 ± 1.0$	1.0 ± 0.0#	0.6 ± 0.0#	3.2 ± 0.0#	15.3 ± 2.4$	0.8 ± 0.0$	0.6 ± 0.0#	0.1 ± 0.0#
HTST	D-Str	18.4 ± 0.5#	0.5 ± 0.0#	0.7 ± 0.0	2.3 ± 0.0#	10.0 ± 0.0	0.8 ± 0.1$	0.8 ± 0.0#	0.1 ± 0.0#
	ND-Str	45.8 ± 0.1#	1.1 ± 0.0#	0.8 ± 0.0	3.1 ± 0.0#	11.0 ± 1.4#	0.8 ± 0.0#	0.7 ± 0.1#	0.1 ± 0.0#

[a]D-Str, freeze-dried strawberry powder + dairy beverage; ND-Str, freeze-dried strawberry powder + distilled water beverage; Unpro, unprocessed; 200-15 and 600-15, 200 and 600 MPa for 15 min holding, respectively; 600-1, 700-1, and 800-1, 600, 700, and 800 MPa for 1 min holding, respectively; HTST, high-temperature−short time (72 °C/20 s). Values represent the mean ± SD; $n = 4$. (*) $P < 0.05$, compared to unprocessed ND-Str; (#) $P < 0.05$, compared to respective unprocessed group (either ND-Str or D-Str); ($) $P < 0.05$, compared to respective HTST group (either ND-Str or D-Str).

Str, from 45.80 ± 0.1 to 29.34 ± 2.1 μg/mL; D-Str, from 18.4 ± 0.5 to 14.22 ± 0.9 μg/mL, $P < 0.05$).

Effect of Processing and Storage on Microbiological Safety. There was no significant difference between TVC measured by automated TEMPO reader and Petrifilm plating ($P > 0.05$) in all beverages samples. There was no detection of potential (strawberry/dairy related) pathogens in all beverage samples. Unprocessed D-NStr beverage samples (TVC at week 0:, 2.05 ± 0.01 $\log_{10}$ CFU/mL) were analyzed for microbial growth over 5 weeks of storage; a significant increase (by 1 log) in TVC after the fifth week was seen compared to the TVC at week 0 ($P < 0.05$). A similar trend of TVC (1 log) was observed after HPP treatment at 200 MPa (15 min) and 600 MPa (1 min) ($P < 0.05$). At the end of 5 weeks of storage, there was a significant increase in TVC values by 1.35 log, 1 log, and 1.1 log for D-NStr beverage samples treated at 600 MPa (15 min), 700−800 MPa (1 min), and HTST respectively, compared to week 0 ($P < 0.05$). There was no detection of TVC in 600 MPa (1 and 15 min), 700−800 MPa (1 min), and HTST-treated ND-Str beverage samples throughout the 5 weeks of storage. For 3 weeks of storage, TVC was not observed in unprocessed and 200 MPa (15 min)-treated ND-Str beverage samples. After 5 weeks, a significant growth in unprocessed (2.5 $\log_{10}$ ± 0.02 CFU/mL) and 200 MPa (15 min) (1.0 $\log_{10}$ ± 0.05 CFU/mL) treated ND-Str beverage samples was observed ($P < 0.05$). Unprocessed D-Str and 200 MPa (15 min)-treated D-Str beverage samples had similar TVC (2.6 $\log_{10}$ ± 0.01 CFU/mL) ($P > 0.05$). HPP treatment of D-Str beverage samples at 600 MPa (1 and 15 min) resulted in a 2 log reduction in microbial counts compared to TVC observed in unprocessed D-Str beverage sample. At 700−800 MPa (1 min) and HTST treatment, no TVC was detected in

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unprocessed D-Str beverage samples. On storage of all D-Str beverage samples at 4 °C, there was a significant increase by 1.5 log in TVC at the end of the 5 week storage period compared to respective week 0 values ($P < 0.05$).

DISCUSSION

The present work was designed to assess the impact of processing on strawberry-containing beverages previously shown to have antioxidant effects in vivo.[6] Moreover, the effect of dairy on the antioxidant capacity of strawberry beverages was investigated, because previous work suggests that dairy interferes with polyphenol-related antioxidant capacity.[10] Our findings indicate that dairy constituents and processing (HPP and HTST) significantly affected the antioxidant capacity and polyphenol content of the strawberry-based beverages. Furthermore, it was observed that HTST processing had a detrimental effect on vitamin C levels compared to unprocessed beverage samples.

Effect of Processing on the Antioxidant Capacity and Anthocyanin in the Strawberry Beverages. Pasteurization processing for extended shelf life products has a number of advantages for consumers and retailers. However, the impact of processing on the nutritive quality of foods has recently gained interest. Traditional pasteurization processes apply heat at relatively high temperatures and have been shown to affect vitamin content.[17] Accordingly, novel processing technologies such as HPP have been developed to minimize heat treatment while still delivering a safe food product. The minimal heat provided by such processing may have important implications on nutrient retention and play a pivotal role in processing foods for preserved or enhanced nutritive value compared to traditional thermal processing.[18]

The advantages and disadvantages of HPP have been comprehensively reviewed.[19] Many of the early studies on HPP emphasized the efficacy of deactivating the microbial load or minimally affecting physicochemical characteristics of food. Research focusing on the effects of HPP on retention of health-promoting phenolic compounds is still in nascent phase. In the present study, we examined the effects of HPP employing various holding times and pressures on antioxidant capacity of strawberry beverages as measured by ORAC and FRAP. Several assays have been used to estimate antioxidant capacities in fresh fruits and vegetables.[13] However, the ORAC assay is said to be more relevant because it utilizes a biologically relevant free radical source.[11] Due to the high pressure in HPP, there is a possibility of forming new compounds or breaking compounds into fragments, and therefore different mechanisms may get different responses after processing. Therefore, in the present study, we utilize the ORAC assay along with the FRAP assay to evaluate the antioxidant capacity of different beverages. ORAC values were unchanged up to 600 MPa (1 and 15 min) in a beverage containing strawberry with no milk (ND-Str) compared to unprocessed beverages; however, ORAC values were increased in HPP beverages containing milk/dairy (D-Str) compared to unprocessed beverages. Several other investigations have also reported that HPP (up to 600 MPa) either elevates or does not influence the antioxidant capacity of foods.[20,21] Increased ORAC values may be associated with volume reduction of beverage samples when treated with HPP[22] or alterations in food matrix leading to release of compounds having free radical binding attributes. In contrast, the reducing ability of strawberry beverages when analyzed by FRAP was significantly reduced in the ND-Str beverage after HPP, but was unchanged in D-Str beverages. HTST and HPP (700−800 MPa for 1 min) significantly decreased the antioxidant capacity as measured by ORAC and FRAP compared to unprocessed beverages. It is observed that HPP demonstrates a variable effect on the polyphenolic compounds in the food matrix.[23] Furthermore, it has been observed that flavonol concentration is reduced by approximately 20% in strawberry product jam in response to high temperatures.[24] Aaby et al. reported that on processing (heat treatment) of strawberry purée, anthocyanin and ascorbic acid contents were decreased with stable ellagic acid derivatives.[25] Correspondingly, in the present study many polyphenolic compounds were significantly reduced as measured by LC-MS/MS in the processed beverage samples (Table 4) at high pressure and temperature conditions. It is possible that polyphenolic compounds are forming complexes with proteins, sugars, and other molecules or are being degraded due to residual enzymatic activities such as peroxidase or polyphenol oxidase (PPO), which therefore reduce their antioxidant activity.[21] Garcia-Palazon et al. reported stability of anthocyanins (pelargonidin-3-*O*-glucoside and pelargonidin-3-*O*-rutinoside) at high pressures (HPP, 800 MPa/18−22 °C/15 min) in red raspberry and strawberry.[26] Future work will be required to determine detailed polyphenolic profile and the stability of strawberry polyphenols in response to different processing and formulation conditions.

Effect of Milk on Antioxidant Capacity and Anthocyanin in the Strawberry Beverages. In the present study, we observed reduced antioxidant capacity when freeze-dried strawberry powder was formulated in a beverage with dairy ingredient (nonfat dry milk powder). Additionally, reduced levels of anthocyanins in the milk-based beverage compared to the water-based beverage were observed, suggesting the possibility of secondary complex formation between polyphenols and milk proteins. It is also possible that polyphenols−protein complexes are retained during the filtration step when samples are prepared for analysis. Zulueta et al. observed that adding milk to orange juice significantly decrease the in vitro antioxidant capacity of the juice (measured by TEAC).[27] Arts et al. demonstrated an interaction between flavonoids and proteins with associated changes in antioxidant capacity in vitro.[28] Polyphenolic compounds possess a high binding affinity for proteins.[29] On the basis of the previous research and observed findings in the present study, we suspect that changes in antioxidant capacity and concentration of phenolic compounds in strawberry−dairy beverages are possibly due to phenolic (tannin)−protein interactions. The significance of these interactions on biological activity remains to be determined. In an in vivo study with blueberries consumed with and without milk, reduced plasma concentrations of phenolic acids (caffeic and ferulic acid) were reported when milk was consumed with the berries, suggesting interference with bioavailability of some phenolic compounds and, possibly, in vivo biological effects.[10]

Effect of Storage on the Antioxidant Capacity and Anthocyanins in the Strawberry Beverages. Fresh strawberries have a short shelf life, mostly due to their high water content and the incidence of microbial load (molds and yeasts). In the present study, the shelf life of strawberry-based beverages after conventional HTST and HPP processing was evaluated on the basis of antioxidant capacity and microbiological end points. At the end of 5 weeks under refrigerated conditions, significantly lower values of ORAC and FRAP were

recorded compared to week 0 ($P < 0.05$) with declines that initiated during week 2, which may be attributed to residual PPO activity as reported by Patras et al.[21] These data are consistent with a study by Ayala-Zavala et al., who showed reduced ORAC of strawberry fruit after storage in temperatures ranging from 0 to 10 °C.[30] Orange juice treated with HPP (500 MPa/5 min/35 °C) had higher ascorbic acid retention compared to conventionally pasteurized (80 °C/30 s) juice after 3 months of storage.[31,32] Sellahewa reported that refrigerated storage (4 °C) of orange juice samples treated with HPP (600 MPa/1 min) for 12 weeks had retained quality factors such as °Brix, viscosity, titratable acidity, and color with retention of ascorbic acid and β-carotene compared to unprocessed orange and juice samples.[33] With reference to the above findings, in the present study there was a significant change in the concentration of pelargonidin-3-*O*-glucoside in strawberry beverage samples at the end of the 5 week storage period compared to week 0 ($P < 0.001$). Microbiological inactivation by HPP in fruits and vegetables has been extensively investigated.[34] Vegetative microorganisms are known to deactivate at the high pressures in the range of 300−600 MPa.[34] In the present study, storage of the HPP-treated strawberry beverage samples (D-Str) at 4 °C for 5 weeks showed 1 log increase in microbial counts.

Significance of Research. The findings from the present study indicate a significant effect of processing and matrix on antioxidant capacity as measured by ORAC, FRAP, and polyphenolic compounds of strawberry-based beverages. However, HPP at pressures ranging from 200 to 600 MPa showed promise as an alternative processing method (to high-temperature processing) for preserving the antioxidant capacity of strawberry−dairy beverage formulations. Future research on fruit−dairy beverages/formulations is required to better understand the interaction of polyphenolic compounds with food matrices and the impact of HPP on antioxidant activities in in vivo systems.

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Author Contributions

B.B.-F., I.E., L.J., and J.C. designed the research approach. R.K.T., K.B., E.P., and I.E. conducted the research. R.K.T., K.B., I.E., and B.B.-F. analyzed the data and compiled the manuscript. All authors were involved with review and the final submitted manuscript. All authors have read and approved the final manuscript.

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Notes

The authors have no conflicts of interest with the present study.

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NOTE ADDED AFTER ASAP PUBLICATION

A calculation error in the original ASAP publication of January 17, 2012, has been corrected in text and graphics with the ASAP posting of April 16, 2012. Figures 1 and 3 were further modified, and a revised version was posted on April 18, 2012.